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(54) Title: LASER TISSUE WELDING WITH DYE ENHANCED SOLDERS

(57) Abstract

A method of welding tissue comprises applying an energy absorbing material such as dye and a soldering agent such as fibrinogen or fibrin glue to tissue to be welded and imparting energy to the energy absorbing material to effect welding of the tissue. Energy can be imparted by directing an energy source such as a laser toward the energy absorbing material, the laser having a dominant wavelength corresponding to a dominant absorption peak wavelength of the dye, so that the dye absorbs energy from the laser, heats the tissue and effects welding of the tissue.

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

Laser Tissue Welding With Dye Enhanced Solders

BACKGROUND OF THE INVENTION

5   The present invention relates to tissue welding with energy
absorbing dye and more particularly to tissue welding using
a source of energy, such as a laser, with energy absorbing
dye and a soldering agent such as fibrinogen applied to the
welding site.

10   Within this application several publications are referenced
by arabic numerals within parentheses. Full citations for
these references may be found at the end of the
specification immediately preceding the claims. The
disclosures of these publications in their entireties are

15   hereby incorporated by reference into this application in
order to more fully describe the state of the art to which
this invention pertains.
       Traditionally, physicians have used suture materials to
appose tissue until the natural healing properties of the

20   body allowed scar formation over the wound. Welding of
tissue with lasers removes the necessity for the use of
sutures, thus allowing primary repair without the creation
of excessive scar and foreign body reaction. Successful
tissue welding using a laser as an energy source has been

25   reported in several surgical fields (1, 2, 9, 21, 22, 23).
Techniques generally rely upon precise primary apposition of
the tissue edges and avoidance of thermal injury to this and
other surrounding tissue. The possibility of early

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disruption due to the diminished strength of welds immediately after creation has prevented more widespread use of this technology.

5   Laser tissue welding techniques have been employed which can produce an anastomosis which causes less turbulence (1), avoids foreign body reaction and granuloma formation, and is faster healing than a conventional sutured anastomosis (2). However, in the event of weld failure, edges of tissue to be

10   approximated are often thermally damaged and cannot be exposed again to laser energy. In addition, although laser welds usually have sufficient strength to withstand systolic blood pressure, they initially are weaker than their sutured counterparts and are more prone to rupture (3, 4).

15   Fibrinogen in conjunction with additional fibrin glue activators, including thrombin and calcium, has been used to enhance microvascular weld strength (5). Laser energy appears to induce protein glue cross-linking (5) and thus creates stronger welds. However, successful application of

20   the laser for tissue welding especially in conjunction with a biologic bonding material requires a high threshold power, typically above 7.6 watts/cm^2, and often results in significant injury to surrounding tissue. It would therefore be advantageous to develop a technique of tissue

25   welding which requires lower laser energy output and which reduces the amount of collateral thermal injury, while still achieving a strong if not stronger weld than obtained using suturing techniques.

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SUMMARY OF THE INVENTION

In accordance with the present invention, a method of
welding a tissue of a subject is provided comprising
5   contacting the tissue to be welded with an energy absorbing
material and a tissue soldering agent in sufficient
quantities to permit a tissue weld to be formed, and im-
parting energy absorbable by the energy absorbing material
to the energy absorbing material in an amount sufficient and
10 under conditions so as to cause heating of the tissue and
welding of the tissue. Preferably, the imparting of energy
comprises imparting electromagnetic energy such as laser
energy to the energy absorbing material. Also preferably,
the contacting of the tissue comprises contacting the tissue
15 with a mixture of energy absorbing material such as dye and
a tissue soldering agent such as fibrinogen. The laser
energy may be from a laser consisting of a phased array of
gallium-alluminum-arsenide semiconductor diodes, or an argon
ion laser, for example. When the laser is the phased array
20 of diodes, the energy absorbing material preferably
comprises indocyanine green (ICG) dye, whereas when the
laser is an argon ion laser, the dye is preferably
fluorescein isothiocyanate (FITC). The soldering agent may
comprise human or animal fibrinogen, but can be any
25 polypeptide. The soldering agent may be a serum protein,
albumin, or fibronectin.
The present invention also provides a tissue soldering
composition for use in welding tissue comprising an energy

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absorbing material and a soldering agent in relative amounts

sufficient to effect welding of tissue when applied to

tissue and when a sufficient amount of energy is imparted to

5   the composition.

By providing an exogenous dye at the welding site, a lower

threshold power can be used, and consequently a smaller

laser. Also, using a smaller laser will result in less

tissue damage. Moreover, the use of a dye enhances

10   selective delivery of energy to target tissue, thus reducing

the amount of collateral thermal injury to exposed tissue in

general. Mixing a soldering agent such as fibrinogen with

the exogenous dye prior to application to a welding site

further enhances the weld strength of the resultant weld.

15   Moreover, use of soldering agents such as fibrinogen

requires neither precise apposition of tissue edges nor

excessive heating of host tissue, thus allowing greater room

for error during the weld. If initial attempts with the

soldering agent fail, reapplication of the soldering agent

20   allows repeated trials without destroying the host tissue.

Bonds created with soldering agents are structurally

stronger than primary laser welds. The fact that the

addition of the laser energy enhancing dye to the soldering

agent, such as fibrinogen, allow selective heating of the

25   solder without injury to underlying host tissue (6) is

especially important for tissue which does not contain

endogenous pigments. Use of exogenous compounds can

photosensitize the solders on the tissue to allow enhanced

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and selective laser energy uptake.   As in photodynamic

therapy or diagnostic fluorescence imaging (10,11,12) a

laser and dye combination can be chosen such that the

5   laser's output frequency matches an absorption peak of the

dye closely, thereby providing efficient and target-specific

laser energy delivery.

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DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, a method of

5   bonding or welding a tissue in a subject is provided
comprising contacting the tissue to be welded with an energy
absorbing material and a tissue soldering agent in
sufficient quantities to permit a tissue weld to be formed,
and imparting energy absorbable by the energy absorbing

10   material to the energy absorbing material in an amount
sufficient and under conditions so as to cause heating of
the tissue and welding of the tissue. Preferably, energy is
imparted by directing an electromagnetic energy source such
as a laser to the energy absorbing material. The contacting

15   of the tissue comprises preferably contacting the tissue
with a mixture of energy absorbing material and a tissue
soldering agent. The energy absorbing material is
preferably a dye. The dye preferably has a dominant
absorption peak at a particular wavelength and the laser

20   preferably has a dominant wavelength corresponding to that
dominant absorption peak of the dye. The energy source also
preferably emits energy having a frequency component in the
visible spectrum to thereby aid in directing the energy
source toward the tissue to be welded. One preferred

25   laser/dye combination is wherein the laser comprises a
phased array of gallium-aluminum-arsenide semiconductor
diodes having a dominant wavelength of about 808 nm and
wherein the dye comprises indocyanine green (ICG). Another

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preferred laser/dye combination is wherein the laser is an
argon ion laser having a dominant wavelength of about 488 nm
and wherein the dye is fluorescein isothiocyanate (FITC).

5   The soldering agent may be human or animal fibrinogen, but
may be any polypeptide. The soldering agent may comprise a
fibrin glue comprising fibrinogen combined with additional
fibrin glue activators such as thrombin, with or without
calcium, as set forth in U.S. Patent No. 4,627,879,

10   incorporated by reference herein. The tissue soldering
agent may comprise a cryoprecipitated suspension comprising
fibrinogen and Factor XIII and a fibrin glue activator
comprising thrombin. The soldering agent may be for example
serum protein, albumin or fibronectin. The laser may be a

15   continuous laser or a discontinuous pulse or chopped laser.
In accordance with another aspect of the invention, a tissue
soldering composition is provided for use in welding tissue
comprising an energy absorbing material and a soldering
agent in relative amounts sufficient to effect welding of

20   tissue when applied to tissue and when a sufficient amount
of energy is imparted to the composition. The composition
may be dye and fibrinogen. The dye may be indocyanine green
(ICG) or fluorescein isothiocyanate (FITC), for example.
The soldering agent may be human or animal fibrinogen, but

25   can be any polypeptide, and may be for example serum
protein, albumin or fibronectin. The composition may also
comprise thrombin and calcium.

MATERIALS AND METHODS

A. INTRODUCTION

Two laser welding studies according to the invention will be
described below. In the first study, the energy source was
a laser diode having a dominant wavelength at about 808 nm
and the energy absorbing material was idocyanine green dye
(ICG) dye having a maximum absorption at about 805 nm. In
the second study, the energy source was an argon ion laser
having a dominant wavelength within the range of 488-514 nm
and the energy absorbing material was fluorescein
isothiocyanate (FITC) dye having a maximum absorption at a
wavelength corresponding closely to the argon laser dominant
wavelength.

B. LASER DIODE/ICG DYE

1. Fibrinogen Preparation

Human fresh frozen plasma was transferred to test tubes and
placed in a freezer at -80° C for at least 12 hours. The
tubes were then thawed at 4° C and centrifuged at 1000 X G
for 15 minutes. The supernatant was decanted leaving the
precipitate, fibrinogen. The viscosity of this solution
allowed easy manipulation with forceps. Fibrinogen can be
stored at -80° C for up to 1 year (7). For further details
regarding the preparation of fibrinogen, attention is
directed to U.S. Patent No. 4,627,879 which is incorporated
by reference herein.

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2. Dye Preparation

Indocyanine green dye (Becton Dickinson, Baltimore, Maryland) was mixed with normal saline to make a saturated
5   solution. The half life of the solution was approximately 10 hours. The dye has a maximum absorption at a wavelength of about 805 nm with an absorption coefficient = 2×10^5 $M^{-1}cm^{-1}$.

3. Laser System

10   Welding was performed with a System 7200 diode laser module coupled into a hand held focusing optic (Spectra-Physics, Mountain View, CA). This laser consists of a phased array of gallium-aluminum-arsenide semiconductor diodes. The major wavelength output of the laser diode is 808 ± 1 nm.
15   Additional bands of laser energy occur in the visible red spectrum and allow the operator to visualize the spot size of the laser during creation of the weld.
The focusing optic allowed for a greater working distance, providing greater visibility of the anastomotic area. With
20   the addition of the focusing optic the beam diameter is 2 mm at a distance of 4 cm. Laser power was measured at the output of the focusing optic.with a Model 201 laser power meter (Coherent Science Division, Palo Alto, CA) and was maintained at 4.8 watts/cm^2. The distance between the
25   focusing lens system optic and welded tissue was maintained at approximately 4 cm during laser application.

4. In vivo Bursting Pressure Measurements

Newly created welds were evaluated in New Zealand White rabbits anesthetized with ketamine (35 mg/kg) and xylazine

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(5 mg/kg). After celiotomy a 16 gauge ridged, duck bill valve vessel cannula (DLP Inc., Grand Rapids, MI) was inserted into the distal abdominal aorta and secured in

5   place with 3-0 silk sutures for measurement of intravascular pressure on a Datascope 2000 pressure monitor (Datascope Corporation, Paramus, NJ). Next, the abdominal aorta was cross-clamped and incised distal to the clamp to create a 7 mm longitudinal aortotomy. Small branch vessels were) Clamping

10   clamped as needed to prevent backflow bleeding. A 6-0 polypropylene suture was placed at each end of the incision and retracted in order to hold the tissue in place during welding. The field was irrigated free of all blood with sterile saline. Fine forceps were used to appose the edges

15   of the aortotomy as needed.

For non-soldered welds (n = 11), the ICG was applied topically to the welding site through a 27 gauge needle immediately prior to laser exposure. For the laser soldered welds (n = 11), a drop of ICG.was mixed with human

20   fibrinogen and then placed with forceps onto the welding site prior to laser exposure. Only the minimum amount of ICG necessary to stain the fibrinogen was used; this allowed precise placement of the dye on the vessel surface. Aortotomies were welded using a power density of 4.8

25   watts/cm^2 for the diode laser. When desiccation of the fibrin glue or tissue was noted, the laser energy was redirected to the next target site. The end point of welding was judged to have taken place when coaptation of the edges was observed and the tissue desiccation and

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retraction had just begun, or either the dye or the fibrin

glue has clotted on the surface and no anastomotic defects

were visible.

5   At the completion of the weld, the branch vessels were

opened and backbleeding into the aorta was permitted for 3

minutes. Next, these branch vessels were clamped again and

an infusion pump was used to advance normal saline through

the previously placed angiocatheter into the distal aorta

10   and the pressure at the time of weld rupture was recorded.

In an additional five rabbits, the above protocol was

followed exactly using the ICG-fibrinogen combination;

however, just prior to measurement of bursting pressure,

25,000 IU of urokinase (Abbott, Chicago, IL) in 0.5cc normal

15   saline was infused into the aorta for five minutes. This

dose of urokinase was the amount needed to completely lyse

0.5cc of clotted rabbit blood within 5 minutes (8).

Bursting pressure was then measured.

5. Control Survival Studies

20   Twenty two New Zealand White rabbits were prepared as above

with a pair of 7 mm longitudinal abdominal aortotomies in

each animal. The superior incision was closed with running

6-0 polypropylene suture and the inferior incision was

welded closed. Each animal underwent ICG enhanced

25   fibrinogen soldering. A threshold power density of 4.8

watts/cm^2 was used for the diode laser. Following the

welds, the inferior aortic cross-clamp was removed. If the

welded anastomosis leaked, the hole was repaired with

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additional fibrinogen and laser exposure. After a leak-free
anastomosis was created, the proximal aortic clamp was
removed and the aorta was examined for evidence of
5   thrombosis or leakage. At intervals between 1 and 90 days,
the animals were sacrificed and weld sites harvested.
Welded tissue was preserved in a 10% formaldehyde solution
buffered to normal pH for HPS, Masson trichrome, or muscle
specific actin staining. In addition, selected specimens
10   were preserved in a 0.5% gluteraldehyde and 2%
paraformaldehyde in 0.05M cacodylate solution (pH 7.38) for
transmission and scanning electron microscopic evaluation.

6. Results

a. In vivo Bursting Pressure Measurements

15   The ICG dye enhanced welds required a laser application time
of approximately one minute to close a 7 mm incision.
Occasionally retreatment was required for residual weld
defects noted after release of the cross-clamps.
Immediately after welding, the bursting pressures of welds
20   created without fibrin glue (262 ± 29 mm Hg, n = 11) were
significantly less than welds soldered with the fibrin glue
(330 ± 75 mm Hg, n = 11)(p less than .05). Suture closures
did not burst at the highest pressures measured, up to 300
mm Hg; however, leakage through needle holes of the suture
25   and small defects in the repair were noted at pressures
above 145 mm Hg. The fibrinogen welds exposed to urokinase
were not significantly weaker than nonperfused fibrinogen
welds (290 ± 74 mm Hg, n = 5)(p=ns).

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b. In vivo ICG Welded Aorta and Suture Control Survival Studies

Among the animals followed for 1 to 90 days, no evidence of anastomotic rupture, thrombosis, or aneurysms occurred in any of the sutured or fibrinogen-ICG solders. Immediately after welding, the applied fibrinogen formed a smooth flow surface onto which red blood cells and platelets adhered. By the second day after surgery, sheets of fibrin were identified crossing the aortotomy weld and the thrombotic response had not increased significantly more than that seen from the specimens harvested immediately after soldering. Within 10 days a new intimal surface had regenerated over the fibrin. By the second week after operation, histiocytes were seen engorged with fibrinogen as the process of resorption started. By the third week after operation, the internal and external elastic lamina remained disrupted, and the initially acellular fibrin was densely packed with proliferating cells which had eosinophilic cytoplasms characteristic of myofibroblasts extending from just below the intimal surface to the adventitia. This identification was confirmed using a Smooth Muscle Specific Actin stain (Enzo Diagnostics, New York, N.Y.) specific for myofibroblasts. These cells appear to have proliferated and laid the matrix for the rapid regeneration of tissue at the weld site. No foreign body reaction was noted. As the healing response continued, fibrin deposited closest to the intimal surface appeared to be resorbed by histiocytes faster than

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fibrin laying on the adventitial surface. By 90 days post

operatively, the fibrin had been almost completely removed

and only normal appearing myointimal cells remained between

5   the external elastic lamina and the intima. Outside the

external elastic lamina, occasional foci of fibrin

surrounded by histiocytes remained; however, in general only

fibrous tissue laid down after the welding was present. No

foreign body reaction was evident.

10   The suture closed aortotomies demonstrated ischemic changes

at the point of suture pressure on the aortic tissue. The

suture closures did not appear to induce thrombus adherence

to any greater extent than the laser welded closures;

however, tissue reaction to the sutures resulted in a

15   fibrotic build-up which distorted the intimal flow surface

by post-operative day 25. Comparable areas of soldered

aorta at the same time after operation demonstrated complete

re-endothelialization, with the aortotomy site completely

covered with intimal cells, and it was difficult to

20   distinguish this site from the surrounding normal rabbit

lumen.

C. ARGON ION LASER/FITC DYE

1. Dye Preparation

Ten milligrams of isomer I FITC (Sigma Inc. St. Louis, MO),

25   10 ml 0.9% sodium chloride irrigation solution at pH 4.5-7

(Abbott Laboratories, Chicago, IL) and 1 ml of 8.4% sodium

bicarbonate at pH 8.4 (Fisher Scientific, Fair Lawn, N.J.)

were mixed, resulting in an orange solution. Two to three

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drops (0.1 cc) of this solution were added topically to the

weld site prior to exposure of the tissue to the argon

laser. In control animals, a similar quantity of 0.9%

5   sodium chloride was applied topically to the weld site.

2. Laser System

Welding was performed with a System 1000 surgical argon

laser with a 1 mm spot size applicator (Coherent Medical

Division, Palo Alto, CA). The laser was operated in the

10   multiline mode. At low laser power the dominant wavelength

is 488 nm.

The applicator was modified by mounting a focusing optic on

the output end of the applicator (Becton Dickenson FACS IV

part No. 53.10006-01, Becton Dickenson Immuno-cytometry

15   Systems, Mountain View, CA). The focusing optic allowed for

a greater working distance, providing greater visibility of

the anastomotic area. With the addition of the focusing

optic the beam diameter is 1.5 mm diameter at 6 cm. Laser

power was measured at the output of the focusing optic with

20   a Model 201 laser power meter (Coherent Science Division,

Palo Alto, CA). The distance between the focusing optic and

the welded tissue was maintained at approximately 6 cm

during laser application.

3. In Vitro Dose-Response Curve

25   The adventitia of freshly harvested rabbit aorta was exposed

to increasing output laser power ranging from 100 to 500 mW

(power fluence range, 5.66 to 12.04 watts/cm^2). For one

group of exposures, a single drop of FITC was topically

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applied to the specimen through a 27 gauge needle. In a
second group, no FITC was used. The tissue was exposed to
the laser system described above for intervals of 15, 30,

5   60, 90, and 120 sec, and gross tissue changes were noted.
The specimens were placed in 10% formalin and stained with
hematoxylin, phloxin, and safranin (HPS).

4. In Vivo Bursting Pressure measurements

Ten New Zealand white rabbits were anesthetized with

10   ketamine (35 mg/kg) and xylazine (5 mg/kg). After celiotomy
a 16 gauge ridged, duck bill valve vessel cannula (DLP Inc.,
Grand Rapids, MI) was inserted into the distal abdominal
aorta and secured in place with 3-0 silk sutures for
measurement of intravascular pressure on a Datascope 2000

15   pressure monitor (Datascope Corporation, Paramus, N.J.).
Next, the abdominal aorta was cross-clamped and incised
distal to the clamp to create a 7mm longitudinal aortotomy.
A 6-0 polypropylene suture was placed at each end of the
incision and retracted to hold the tissue in place during

20   welding. The field was irrigated free of all blood with
sterile saline. Fine forceps were used to oppose the edges
of the aortotomy as needed. For the FITC welds, the dye was
topically applied to the welding site through a 27 gauge
needle. Only the minimum amount of FITC necessary to stain

25   the welded area was used. Although it was impossible to
control precisely the lateral spread of the dye once applied
to the tissues, one to two drops of dye were usually
adequate. Aortotomies were welded using power densities of

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3.8 watts/cm^2 with FITC and 7.6 watts/cm^2 without FITC.
These power densities represented the minimum power levels
at which reliable welds could be consistently obtained.

5 When desiccation of the aorta was noted, the laser energy
was redirected to the next target site. The end point of
welding was judged to have taken place when coaptation of
the edges was observed and the tissue desiccation and
retraction had just begun.

10 At the completion of the weld, an infusion pump was used to
advance normal saline through arterial pressure tubing and
the previously placed angiocatheter into the distal aorta.
To minimize the number of animals sacrificed, we were able
to perform two to three welds and bursting pressure

15 determinations on the aorta of each animal by progressively
moving the aortic cross clamp distally after each bursting
pressure determination.

5. In Vivo welded Aorta and Suture Control Survival Studies

Twenty-five additional New Zealand White rabbits were

20 prepared as above with a pair of 7 mm longitudinal abdominal
aortotomies in each animal. The superior incision was
closed with running 6-0 polyproylene suture, and the
inferior incision was welded closed. Eighteen animals
underwent FITC-enhanced welding; seven rabbits underwent

25 laser welding without addition of FITC. A threshold power
density of 3.8 watts/cm^2 was used for the non-FITC welds.
Following the welds, the inferior aortic cross-clamp was
removed. If the welded anastomosis leaked, the hole was

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repaired by additional laser exposure. After a leak-free

anastomosis was made, the proximal aortic clamp was removed

and the aorta was examined for evidence of thrombosis or

5   leakage. At intervals between 1 and 75 days, the animals

were sacrificed and weld sites harvested. Welded tissue was

preserved in a 10% formaldehyde solution buffered to normal

pH for PHS or Masson trichrome staining.

6. Results

10   ### a. In Vitro Dose-Response Curve

For FITC stained in vitro rabbit aorta, the threshold for

tissue blanching was 15 sec of 100 mW exposure. As the time

of exposure and laser power density increased, the tissue

acquired a reddish hue followed by further desiccation,

15   contraction, and eventual charring at approximately 30 sec

of 300 mW exposure. No holes were created until 90 sec of

exposure to 400 mW laser energy. In the unstained aorta, no

tissue effects were observed until 15 sec of 300 mW laser

energy was applied. The initial tissue desiccation rapidly

20   progressed to charring, and by 90 sec of exposure to this

power, a hole had been created. The progression of tissue

changes in the FITC-stained group in comparison to the

unstained controls is demonstrated in Table 1 reproduced at

the end of this specification. As compared with

25   FITC-stained aorta, three times greater laser energy was

needed to induce the same tissue changes on nonstained

specimens. Also, the progression of tissue changes from

initial tissue effects of the laser to charring and eventual

hole creation was more gradual in tissue stained with FITC.

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b. In Vivo Bursting Pressure Measurements

Both FITC and non-FITC welds required laser application time of approximately 90 sec to close a 7 mm incision.

5 Occasionally a retreatment was required for residual weld defects noted after release of the cross-clamps. Mean bursting pressures immediately after weld creation were 164 mm Hg for FITC group compared with 147 mmHg for the non-FITC-welded group. Suture closures did not burst at the

10 highest pressure used (300 mm Hg); however, leakage through needle holes of the suture and small defects in the repair were noted at pressures above 125 mm Hg.

c. In Vivo FITC-Welded Aorta and
Suture Control Survival Studies

15 Among the animals followed for 1 to 75 days, no evidence of anastomotic rupture, thrombosis, or aneurysms occurred in any of the suture or FITC welds. The weld site of FITC-enhanced argon ion laser welds was characterized by myointimal proliferation with a regenerated intimal surface.

20 Collagen deposit was noted at the weld site. One of the welds performed without FITC developed a small anastomotic dilation by 2 weeks postoperatively. No other abnormalities were noted in this group. Histologically, carbonaceous debris was usually visible on the surface tissue directly

25 exposed to the argon energy without FITC. After treatment without FITC, the argon ion laser induced a 50-100 um region of amorphous collagen surrounding the point of welding. A 300 um region of tissue manifested the loss of nuclear detail and cellular integrity characteristic of thermally

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damaged areas. No deposition of carbonaceous debris was

evident on the FITC weld specimens. The suture closed

aortotomies demonstrated ischemic changes at the point of

5   suture pressure on the aortic tissue. These closures did

not appear to induce thrombus adherence to any greater

extent that the laser-welded closures. By 2 weeks after

laser welding, a new endothelial surface had repopulated the

intimal surface. Similar changes had occurred in the

10   suture-closed aortotomies, although patches of denuded

endothelium often remained.

D. DISCUSSION OF STUDIES

All three elements in the described welding system i.e. an

energy source, an energy absorbing material and a tissue

15   soldering agent meet the requirements of simplicity and

safety for application in the clinical setting. Idocyanine

green, fibrin glue, and diode lasers have been proven safe

in clinical application (8,14,15). Indocyanine green has a

large absorption coefficient at the 808 nm output of the

20   diode laser. The diode laser is an inexpensively

manufactured solid state semiconducting electronic device

with heretofore limited medical applications due to low

energy output capacities (less than 400 mW). When combined

with topically applied indocyanine green dye, lasers at this

25   wavelength can cause substantial and rapid tissue effects.

The diode laser, for example, because of its low cost, small

size, simple power requirements and durability, will replace

other solid state lasers and popular gas discharge lasers in

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many applications. Finally, fibrin glue has been used as a
hemostatic agent without significant complications. In
elective surgery, fibrinogen can be harvested from the

5   subject. Addition of thrombin and calcium have been
required to achieve hemostasis intra-operatively (16,17).
We were unable to create durable anastomoses using this
clotting system. Following laser exposure, however, a
durable fibrin coagulum is achieved even without the use of

10   thrombin and calcium. Laser "spot welding" may be useful
for repair of anastomotic leaks as well as during creation
of sutured anastomoses.

We have observed that argon ion laser welding of biliary
tissue is facilitated by the bile and blood staining of the

15   tissues [6]. Here we employ an exogenous compound to
photosensitize vascular tissue for laser welding. Under
identical experimental conditions, the use of FITC enabled
welds to be formed at one-half the threshold laser power
density as compared with non-FITC welds.

20   The threshold laser power density for welding without FITC
is in agreement with previous reports (22). By using FITC,
this threshold can be decreased. The lower energy required
for welding minimized thermal damage to surrounding, healthy
tissue. Additionally, because of the topical application of

25   FITC, the energy deposition is localized to the area of the
weld. The circumscribed zone of coagulation injury was
apparent on the adventitial surface of the vessel without
underlying thermal damage.

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The specificity of energy deposition is further enhanced by use of argon ion laser power outputs below 0.2 watts. In this range, an all-lines mode argon laser energy output is

5   predominantly a 488 nm wavelength. This wavelength closely corresponds to the maximum absorption for FITC. At higher powers other wavelengths contribute to the total output and can be nonspecifically absorbed by surrounding tissue, leading to thermal injury.

10   Blood also appeared to act as an engaging endogenous chromophore. Welds were easier to create and appeared more durable if the aortic tissue was blood stained. In the bursting strength experiments, total elimination of blood from the field by copious irrigation prior to welding made

15   welding without FITC much more difficult. With use of FITC, the lack of blood in the field was much less important. The FITC dye also helps to improve visualization of the weld site because of its bright green fluorescence as observed through standard argon ion laser safety goggles used to

20   filter out laser light (Laserguard, Glendale, CA). Extrinsic dye to enhance selective delivery of laser energy to tissue has been used in photoradiation of cancers, ophthalmologic vessel ablation, and diagnostic fluorescence imaging (10, 11, 12, 24). Use of exogenous dyes on target

25   tissues may prove superior to use of endogenous chromophores by allowing greater control of local chromophore concentration and energy delivery.

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A variety of mechanisms may be responsible for fibrinogen
soldering. The inability of urokinase to prevent welding
with fibrin glue suggests that enhanced clotting of rabbit
5   blood with exogenous fibrinogen is not the dominant
mechanism of soldering. More likely, heated fibrinogen
undergoes covalent crosslinking independent of the
coagulation cascade and is transformed into an insoluble,
durable biologic glue holding together edges of the host
10 tissue. The amorphous coagulum is rapidly infiltrated with
myofibroblasts and covered with host endothelial cells.
Fibrin may be stimulating this rapid regeneration of vessel
wall (18). As the fibrin glue is resorbed by histiocytes,
myointimal proliferation continues until a new media has
15 been created. The internal and external elastic lamina, on
the other hand, are not reconstituted. As has been reported
by others (19,20), no foreign body reaction is evident. In
effect, the fibrin solder consists of a biodegradable
scaffold upon which the divided vessel heals. Use of dye
20 enhanced fibrinogen also reduces injury to tissue
surrounding the weld and may allow more rapid proliferation
of myofibroblasts and the deposition of the extracellular
matrix necessary for regeneration of the vascular tissue at
the welding site. The foreign body response to suture
25 material (19) and the irregular flow surface caused by the
sutures are avoided. Other advantages include superior
bursting strength, less red blood cell death and less plaque
adherence to the intimal vessel wall. As our techniques

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have improved, the laser soldering method has also proven
faster than sutured and conventional laser weld anastomoses,
especially in smaller sized vessels.

5   Despite theoretical advantages, laser tissue welding has
been hindered by technical obstacles and an inability to
achieve consistent bonding. Use of dye enhanced protein
solders obviates these difficulties and make clinical
applications of laser welding technology feasible.

10   In the case of the 808 nm diode laser energy output, the
output wavelength lies within the "optical window" of the
vessel wall (13), thus failing to show any tissue effects
even at the highest energy outputs available (9.6
watts/cm^2). In this way, despite elevation of fibrinogen

15   temperature during welding, the underlying vessel wall is
not damaged. The solder-dye mix also provides a convenient
means to apply the dye in a controllable fashion.
While two different laser/dye combinations have been
described, namely an 808 nm wavelength near infrared diode

20   laser with indocyanine green (ICG) dye, and an argon ion
laser with fluorescein isothiocyanate (FITC) dye, other
energy sources and energy absorbing materials may be used.
The energy source need not be a laser, but could be any type
of energy source such as for example an X-ray source,

25   radiowave source, microwave source or non-coherent visible
light source. The energy absorbing material need not be a
dye, but could be any energy absorbing substance in any form
which is used to absorb energy from energy sources. As in

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photodynamic therapy or diagnostic flourescence imaging, a

laser and dye combination can be chosen such that the

laser's output wavelength matches the absorption peak of the

5   dye, thereby providing efficient and target-specific laser

energy delivery.

In the case of the 808 nm diode laser the output wavelength

lies within the "optical window" of the vessel wall. That

is, the laser fails to show any tissue effects even at the

10   highest power outputs available (9.6 watts/cm^2). In this way

despite elevation of fibrinogen temperature during welding,

the underlying vessel wall is minimally damaged. The

soldering agent-dye mix also provides a convenient means to

apply the dye in a controlled manner.

15   Other dye/laser combinations which have been successfully

tested are FITC-dextran/argon ion laser, methylene blue,

acridine orange, or rose bengal/dye laser, and Kodak

Q-switch II/Nd:Yag (1064 nm) laser. Other possible

dye/laser combinations include, for example, hematoporphyrin

20   derivative/argon ion laser and tetramethyl rhodamine

isothiocyanate/krypton ion laser.

It is preferable to use a dye that is water soluble, such as

the FITC dye disclosed above. The dye and energy absorbing

material are preferably mixed before application to the

25   tissue; otherwise the dye will tend to run freely to areas

which are not desired, such as inside an anastomosis.

The soldering agent may be any substance which after

exposure to the energy source above, especially in

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conjunction with one of the energy absorbing materials, is
transformed into a biological glue which will hold tissue
together. The solders may be synthetic or naturally occur-
5   ring, biodegradeable or permanent, and may require combina-
tion with solvents and gels in order to acquire the appro-
priate consistency for the welding process. In effect, the
solder constitutes a biodegradable scaffold upon which the
divided tissue heals. Use of dye enhanced solders also
10   reduces injury to tissue surrounding the weld and may allow
more rapid proliferation of myofibroblasts and the
deposition of the extracellular matrix necessary for
regeneration of the vascular tissue at the weld site. The
foreign body response to suture material and the irregular
15   flow surface caused by the sutures are avoided.
The soldering agent may have as a base composition human or
animal proteins, such as bovine proteins. Serum proteins
such as albumin or fibronectin may also be used. The tissue
to be welded may already have a natural soldering agent such
20   as collagen or elastin. These and other additives may
change the viscosity of the overall soldering agent
composition and make it easier to apply.
One may also wish to add one or more drugs to the energy
absorbing material/soldering agent mixture so that the
25   mixture may also serve as a slow release drug delivery
system. One such drug may be a growth factor to improve
wound and tissue healing.

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The present invention provides new means of apposing tissue
to allow more natural healing. The techniques developed are
quicker and in some instances safer than sutured repair.

5   The potential applications of the present invention include:
(1) primary tissue soldering, especially in vascular, colon,
fallopian tube, tracheal, esophagus and neural anastomoses,
(2) repair of sutured anastomoses when leakage occurs,
especially in cardiovascular surgery when the protein's

10   clotting mechanism is disrupted, (3) management of oozing
surfaces, for example injured liver, renal, or splenic
tissue following trauma, (4) repair of organs and endoth-
elial layers within said organs, such as a colon lining, and
(4) secondarily to strengthen a sutured area. Additional

15   uses for the invention will occur to those skilled in the
art and will become more widespread once the present
technique becomes more widely available.
The method according to the present invention can be
practiced on any living subject, human or animal. The

20   preferred subject is a human being undergoing surgical
operations.
The relative quantities of the energy absorbing material and
soldering agent which contact the tissue should be
sufficient to the effect welding of the tissue. It is

25   possible that the tissue already has a natural soldering
agent present so that such agent need not be added
exogenously.

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Contacting the tissue with a soldering agent thus includes presence of a soldering agent endogenously and/or adding a soldering agent exogenously.

5   Similarly, the energy imparted to the energy absorbing material should be sufficient to effect heating of the tissue to result in welding. For example, when the energy is being imparted using a laser, factors such as the temperature and size of the tissue area to be welded will

10 vary, and therefore the amount of energy absorbing material, soldering material and energy level of the laser required should necessarily take these factors into account.
    As used herein, the term welding includes welding in the conventional sense, as well as bonding and soldering.

15 Although a preferred embodiment of the invention has been . disclosed and described herein, it should be understood that the present invention is in no sense limited thereby and its scope is to be determined only by that of the appended claims.

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TABLE 1. Dose-Response Curve*

Milliwatts	Seconds				
	15	30	60	90	120
Argon laser with FITC					
100	1	2	2	2	2
200	2	2	2	2	2
300	3	4	4	4	4
400	4	4	4	5	5
500	5	5	5	5	5
Argon laser without FITC					
100	0	0	0	0	0
200	0	0	0	0	0
300	3	3	4	5	5
400	5	5	5	5	5
500	5	5	5	5	5

*0, no observable effect; 1, blanching; 2, red; 3, curling
of tissue after dessication; 4, carbonaceous deposition; 5,
perforation

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REFERENCES

1.   White RA, Kopchok G, Donayre C, White G, Lyons R, Fujitani R, Klein SR, Uitto J: Argon laser-welded arteriovenous anastomoses. J Vasc Surg 1987; 6:447-53.

2.   Oz MC, Bass LS, Popp HW, Chuck RS, Johnson JP, Trokel SL, Treat MR: In vitro comparison of THC:YAG and argon ion lasers for welding of biliary tissue. Lasers Surg Med 1989; 9:248-253.

3.   Basu S, Wang S, Robertazzi R, Grubbs PE, Jacobowitz I, Rose D, Acinapura AJ, Cunningham JN Jr: In vitro bursting strength studies of laser-welded tissue and comparison with conventional anastomosis. J Vasc Surg 1988;7:420-2.

4.   Quigley MR, Bailes JE, Kwaan HC, Cerullo LJ, Brown JT, Fitzsimmons J: Comparison of bursting strength between suture-and laser-anastomosed vessels. Microsurgery 1985;6:-229-232.

5.   Grubbs PE Jr., Wang S, Marini C, Basu S, Rose DM, Cunningham JN: Enhancement of CO_2 laser microvascular anastomoses by fibrin glue. J Surg Res 1988;45:112-119.

6.   Oz MC, Chuck RS, Johnson JP, Parangi S, Bass LS, Nowygrod R, Treat MR. Photosensitizer enhanced diode laser vascular welding. Surg Forum 1989 (in press).

- 31 -

7.   Gestring GF, Lerner R: Autologous fibrinogen for tissue
adhesion, hemostasis, and embolization.   Vasc Surg 1983;17:-
294-304.

8.   Pang D, Sclabassi RJ, Horton JA: Lysis of intraven-
tricular blood clot with urokinase in a canine model: part
2. Neurosurg 19:547, 1986.

9.   Poppas DP, Schlossberg SM, Richmond IL, Gilbert DA,
Devine CJ: Laser welding in urethral surgery: Improved
results with a protein solder. J Urology 1988;139:415-417.

10.   Dougherty TJ, Wieshaupt KR, Boyle DG: Photoradiation
therapy of human tumors. In Regan JD, Parrish JA eds.   "The
Science of Photomedicine." New York: Plenum Press, 1982:
625-638.

11.   Prout GR, Lin CW, Benson R, Nseyo UO, Daly JJ, Griffen
PP, Kinsey J, Tian M, Lao Y, Mian Y, Chen X, Ren F, Qiao S:
Photodynamic therapy with hematoporphyrin derivative in the
treatment of superficial transitional cell carcinoma of the
bladder. NEJM 1987;317:1251-1255.

12.   Wolfe DR: Fluorescein angiography basic science and
engineering. Ophthalmology 1986;93:1617-1620.

SUBSTITUTE SHEET

- 32 -

13. Anderson RR, Parrish JA: Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. Science 1983;220:524-7.

14. Benchimol A, Li Y, Legler JF, Dimond EG: Rapidly repeated determinations of the cardiac output with the indicator-dilution technic. Am J Card 1964;13, 790-797.

15. Puliafito CA, Deutsch TF, Boll J, To K: Semiconductor laser endophotocoagulation of the retina. Arch Opthalmol 1987;105:424-427.

16. Dresdale A, Rose EA, Jeevanadum V, Reemtsma K, Bowman FO, Malm JR: Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery 1985;97:750-754.

17. Wolner E. Fibrin gluing in cardiovascular surgery: Thorac Cardiovasc Surgeon 1982;30:236-237.

18. Powanda MC, Moyer ED: Plasma proteins and wound healing. Surg Gynecol Obstret 1981;153:749-755.

19. Pearl RM, Wustrack KO, Harbury C, Rubenstein E, Kaplan EN: Microvascular anastomosis using a blood product sealant-adhesive. Surg Gynecol Obstet 1977;144:227-231.

SUBSTITUTE SHEET

20. Baker JW, Spotnitz WD, Matthew TL, Fechner RE, Nolan SP: Mediastinal Fibrin glue: hemostatic effect and tissue response in calves.   Ann Thorac Surg 1989;47:450-452.

21. White RA, Kopchok G, Donayre C, Abergel RP, Lyons R, Klein SR, Dwyer RM, Uitto J: Comparison of Laser-welded and sutured arteriotomies. Arch Surg 1986; 121:1133-1135.

22. White RA: Technical frontiers for the vascular surgeon: laser anastomotic welding and angioscropy-assisted intraluminal instrumentation. J. Vasc. Durg. 1987; 5(4):673-680.

23. White FV: Laser tissue repair with the CO_2 laser. Proc SPIE 1989; 1066 (in press).

24. Ohnishi Y, Yamana Y, Ishibashi T: Effect of argon laser photoradiation on monkey retina treated with hematoporphyrin derivative-Fluorescein angiographic and light microscopic study. Jpn J Ophthalmol 1987; 31:160-179.

25. Oz MC, Chuck RS, Johnson JP, Parangi S, Bass LS, Nowygrod R, Treat MR: Indocyanine Green Dye Enhanced Vascular Welding   With the New Infrared Diode Laser, Vasc. Surg. 1989 (in press)

26. Chuck RS, Oz MC, Delohery TM, Johnson JP, Bass LS, Nowygrod R, Treat MR: Dye-Enhanced Laser Tissue Welding, Lasers Surg. Med. 1989 (in press)

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What We Claim Is:

1.   A method of welding a tissue of a subject
comprising:

5   contacting the tissue to be welded with an energy
absorbing material and a tissue soldering agent in
sufficient quantities to permit a tissue weld to be formed;
and

           imparting energy absorbable by the energy
10   absorbing material to the energy absorbing material in an
amount sufficient and under conditions so as to cause
heating of the tissue and welding of the tissue.

2.   The method according to claim 1, wherein the
imparting of energy comprises imparting electromagnetic
15   energy to the energy absorbing material.

3.   The method according to claim 1, wherein the
contacting of the tissue comprises contacting the tissue
with a preformed mixture of energy absorbing material and a
tissue soldering agent.

20   4.   The method according to claim 1, wherein the
imparting of energy comprises imparting laser energy to the
energy absorbing material.

5.   The method according to claim 1, wherein the
contacting of the tissue comprises contacting the tissue
25   with a dye.

6.   The method according to claim 1, wherein the
contacting of the tissue comprises contacting the tissue
with a dye having a dominant absorption peak at a particular

wavelength and wherein imparting of energy comprises

imparting laser energy having a dominant wavelength

corresponding to the dominant absorption peak of the dye.

5   7.   The method according to claim 1, wherein the

imparting of energy comprises imparting of energy having a

frequency component in the visible spectrum to the energy

absorbing material.

           8.   The method according to claim 1, wherein the

10   imparting of energy comprises imparting energy having a

dominant wavelength of about 808 nm to the energy absorbing

material.

           9.   The method according to claim 8, wherein the

contacting of the tissue comprises contacting the tissue

15   with a mixture of indocyanine green (ICG) dye and a

soldering agent.

           10.   The method according to claim 1, wherein the

imparting of energy comprises imparting of energy having a

dominant wavelength of about 488 nm to the energy absorbing

20   material.

           11.   The method according to claim 10, wherein the

contacting of the tissue comprises contacting the tissue

with a mixture of fluorescein isothiocyanate (FITC) dye and

a soldering agent.

25   12.   The method according to claim 1, wherein the

contacting of the tissue comprises contacting the tissue

with a soldering agent comprising human or animal

fibrinogen.

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13. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising fibrin glue which

5   comprises fibrinogen combined with at least one fibrin glue activator.

14. The method according to claim 13, wherein the fibrin glue activator comprises thrombin.

15. The method according to claim 13, wherein the

10   fibrin glue activator comprises calcium.

16. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a tissue soldering agent comprising a cryoprecipitated suspension which comprises fibrinogen and Factor XIII, and a

15   fibrin glue activator comprising thrombin.

17. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising a polypeptide.

18. The method according to claim 1, wherein the

20   contacting of the tissue comprises contacting the tissue with a soldering agent comprising a serum protein.

19. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising albumin.

25   20. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with a soldering agent comprising fibronectin.

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21. The method according to claim 1, wherein the imparting of energy comprises imparting laser energy continuously to the energy absorbing material.

22. The method according to claim 1, wherein the imparting of energy comprises imparting laser energy discontinuously to the energy absorbing material.

23. The method according to claim 1, wherein the contacting of the tissue comprises contacting the tissue with an energy absorbing material and tissue soldering agent applied into or on the surface of the tissue.

24. A tissue soldering composition for use in welding tissue comprising an energy absorbing material and a soldering agent in relative amounts sufficient to effect welding of the tissue when applied to the tissue and when a sufficient amount of energy is imparted to the composition.

25. A tissue soldering composition according to claim 24, comprising dye and fibrinogen.

26. A tissue soldering composition according to claim 24, wherein said energy absorbing material is indocyanine green (ICG) dye.

27. A tissue soldering composition according to claim 24, wherein said energy absorbing material is fluorescein isothiocyanate (FITC) dye.

28. A tissue soldering composition according to claim 24, wherein said soldering agent comprises human or animal fibrinogen.

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29. A tissue soldering composition according to claim 24, wherein said soldering agent comprises a polypeptide.

30. A tissue soldering composition according to claim
5   24, wherein said soldering agent comprises a serum protein.

31. A tissue soldering composition according to claim 24, wherein said soldering agent comprises albumin.

32. A tissue soldering composition according to claim 24, wherein said soldering agent comprises fibronectin.

10   33. A tissue soldering composition according to claim 24, wherein said soldering agent comprises thrombin and calcium.
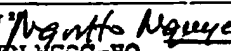

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05125

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC 5: A61N 5/06 A61B 19/00;
US: 128/ 343, 397, 398, 898; 606/8

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System	Classification Symbols
US	606/8
	128/395, 397, 398, 898

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	Lasers in surgery and medicine Vol. 9, Vol. May, 1989, Saur, "The first sutureless End-to-End Bowel Anastomosis" pages 70-73	1,2,4,5,12-14 17,18,20,21,23-25 28-30,32,33
X	The Journal of Urology; Vol. 139, February 1988, Poppos et al, :Laser Welding in Urethane Surgery: Improved Results with a protein solder" pages 415-417	1-6,12-20,23, 24, 28-33
X	Journal of Surgical Research, Vol. 45, Vol. July 1988, Grubbs, Jr. et al, "Enhancement of CO_2 Laser Anastomoses by Fibrin Glue" pages 112-119	1-16,12-18,20, 22-25,28-30,32, and 33
		5-11, 21-23,26 27

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ⁸
30 November 1990	20 FEB 1991
International Searching Authority ¹	Signature of Authorized Officer ²⁰
ISA/US	David Shay NGUYEN NGCC-HO INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET		
Y	US,A 3,769,963 (Goldman)06 November 1973 column 2 lines 10-32	5-11,21-23,26, 27
Y	US,A 4,774,339 (Haugland) 27 September 1988 see column 1, lines 51-65	5,6,10,11,21-23 27
Y	US,A 4,805,623 (Jobsis) 21 February 1989 see column 23, lines 6-9	5-9,26
Y	US,A 4,457,992 (Bhattacharjee) 03 July 1984 see column 5, lines 40-45 and column 6, lines 30-33	5-9,26
Y	Journal of Vascular Surgery, Vol. 6, No. 5, November 1987, White et al, "Argon laser-welded arteriovenous anastomoses" pages 447-453	5-9,10,11,21-23 26,27

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers   . because they relate to subject matter ¹ not required to be searched by this Authority, namely:

2.☐ Claim numbers .... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹, specifically:

3.☐ Claim numbers_____ , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims: it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 4-90)